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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : B01L 3/00, 11/00

A1

(11) International Publication Number: WO 98/56505

(43) International Publication Date: 17 December 1998 (17.12.98)

(21) International Application Number: PCT/US98/12127

(22) International Filing Date: 8 June 1998 (08.06.98)

(30) Priority Data:
08/871,338 9 June 1997 (09.06.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 08/871,338 (CIP)
Filed on 9 June 1997 (09.06.97)

(71) Applicant *(for all designated States except US)*: CALIPER TECHNOLOGIES CORPORATION [US/US]; 1275 California Avenue, Palo Alto, CA 94028 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: PARCE, J., Wallace [US/US]; 754 Los Robles Avenue, Palo Alto, CA 94303 (US). KOPF–SILL, Anne, R. [US/US]; 30 Minoca Road, Portola Valley, CA 94028 (US).

(74) Agents: QUINE, Jonathan, A. et al.; Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111–3834 (US).

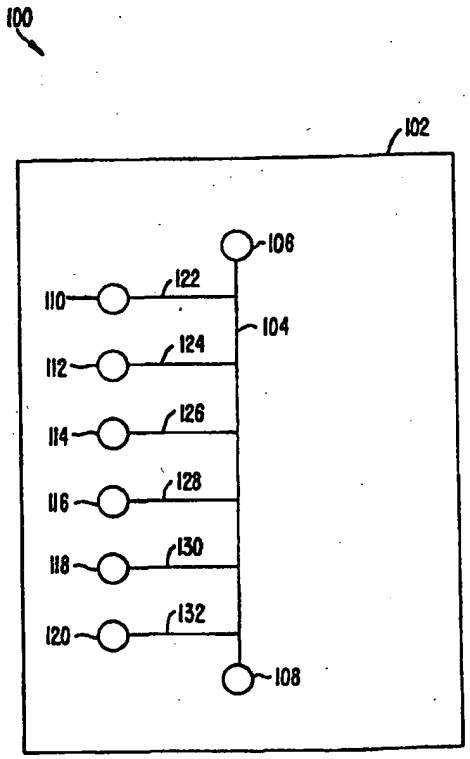
(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: METHODS AND APPARATUS FOR *IN SITU* CONCENTRATION AND/OR DILUTION OF MATERIALS IN MICROFLUIDIC SYSTEMS

(57) Abstract

The present invention is generally directed to methods, apparatus and systems for use in performing *in situ* dilution or concentration of a particular subject material in a microfluidic device or system. These methods and apparatus may generally be integrated with other microfluidic operations and/or systems, to perform a number of different manipulations, wherein dilution or concentration, carried out within the context of the microfluidic device or system, is just one part.



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METHODS AND APPARATUS FOR *IN SITU* CONCENTRATION AND/OR DILUTION OF MATERIALS IN MICROFLUIDIC SYSTEMS

BACKGROUND OF THE INVENTION

Carrying out chemical or biochemical analyses, syntheses or preparations, even at the simplest levels, requires one to perform a large number of separate manipulations on the material components of that analysis, synthesis or preparation, including measuring, aliquoting, transferring, diluting, concentrating, separating, detecting etc.

In developing microfluidic technologies, researchers have sought to miniaturize many of these manipulations and/or to integrate these manipulations within one or a few microscale devices. Many of the above described manipulations easily lend themselves to such miniaturization and integration. For example, the use of these microfluidic technologies has been described in a number of applications, including, e.g., amplification (U.S. Patent Nos. 5,587,128 and 5,498,392) and separation of nucleic acids (Woolley et al., Proc. Nat'l. Acad. Sci. 91:11348-352 (1994) and hybridization analyses (WO 97/02357 to Anderson).

Despite the application of microfluidic technologies to these manipulations, there are still a number of areas where that application is not so easily made. For example, the performance of large dilutions generally requires the combination of a small volume of the material that is desired to be diluted with a large volume of diluent. By definition, microfluidic systems have extremely small overall volumes, and are typically unable, or less able, to handle the larger volumes required for such dilutions. Further, such large dilutions also typically require the accurate, repeatable dispensing of extremely small volumes of the material to be diluted. However, most microfluidic technology is incapable of accurately dispensing fluid volumes substantially less than a microliter. Although the problems associated with the inability to aliquot extremely small volumes might generally be overcome by performing serial dilutions, such serial dilutions generally require devices with substantially larger volumes, e.g., tens or hundreds of microliters. Specifically, even if one assumes a lower limit of fluid handling of 100 nanoliters, a 1:10 dilution would require a

device to handle at least a volume of 1 µl. Further serial dilution steps only increase the required volume.

It would therefor be desirable to provide microfluidic systems which are capable of performing each of the various manipulations required, and which are capable of doing so with a sufficiently small volume whereby, multiple operations can be integrated into a single low volume device or system and performed automatically and with a high degree of precision. Of particular interest would be a microfluidic device or system, as well as methods for using such devices and systems for performing *in situ* dilution or concentration of a particular material within a microfluidic format. The present invention meets these and many other needs.

SUMMARY OF THE INVENTION

The present invention is generally directed to methods, apparatus and systems for performing *in situ* concentration or dilution of a material in microfluidic devices or systems. In one aspect, the present invention provides microfluidic devices and systems for performing *in situ* dilution, and particularly *in situ* serial dilution, of a particular subject material. The devices and/or systems typically comprise a microfluidic device which has at least one main channel disposed therein, where the main channel has at least one microscale cross-sectional dimension. The devices and/or systems also typically comprise at least a first source of the subject material that is to be diluted, in fluid communication with the main channel at a first point along the length of the channel, at least a first source of diluent in fluid communication with the main channel at a second point along the length of the channel, and at least a first reservoir in fluid communication with the main channel at a third point along the length of the channel. The systems of the present invention further comprise a fluid direction for delivering diluent to the main channel to be combined with the subject material to form first diluted material, and for removing at least a portion of the first diluted material from the main channel to the reservoir. Additional diluent sources and reservoirs also may be supplied to further dilute the subject material.

In a closely related aspect, the present invention provides a microfluidic system for continuously diluting a subject material within a microfluidic device. The devices and/or systems typically comprise a microfluidic device which has at least one main channel disposed therein, where the main channel has at least one microscale cross-sectional dimension. The device also typically comprises at least a first source of the subject material in fluid communication with the main channel at a first point along the

length of the channel, at least a first source of diluent in fluid communication with the main channel at a second point along the length of the channel, and at least a first reservoir in fluid communication with the main channel at a third point along the length of the channel. The systems of the present invention further comprise a fluid direction system for continuously delivering diluent to the main channel to be combined with the subject material to form first diluted material, and continuously transporting a portion of the first diluted material from the main channel to the reservoir.

The present invention also provides microfluidic systems for *in situ* concentration of a subject material within a microfluidic device. In this aspect, the system comprises a microfluidic device having a first channel disposed therein, which channel has first, second and third fluid regions disposed therein. The first fluid region typically comprises the subject material and has a first conductivity, whereas the second and third fluid regions are disposed within the first channel on both ends of the first fluid region. The second and third fluid regions have a second conductivity, where the second conductivity is greater than the first conductivity. The system also typically comprises an electroosmotic fluid direction system for transporting the first and second fluid regions along the first channel.

In a further aspect, the present invention provides methods for *in situ* dilution of a subject material in a microfluidic device. The methods typically comprise combining a first volume of the subject material with a first volume of diluent in a first microscale channel to form a first diluted material. At least a first portion of the first diluted material is then transported out of the first channel. A second volume of diluent from a second diluent source is then delivered to the first microscale channel to combine the second volume of diluent with a second portion of the first diluted material to form a second diluted material.

In a related aspect, the present invention provides a method for *in situ* dilution of a material in a microfluidic device, which method comprises combining a first volume of said material with a first volume of diluent in a first region of a microfluidic device to form a first diluted material. A portion of the first diluted material is then transported into a second region of the microfluidic device, i.e., a reservoir, where it is combined with a second volume of diluent to form a second diluted material.

In a further aspect, the present invention provides a method for the *in situ* concentration of a material in a microscale channel. The method comprises introducing a first fluid containing the material into a microscale channel to provide a first fluid region

within the channel. The first fluid has a first conductivity, and is bounded by second and third fluid regions, where the second and third fluid regions have a second conductivity which is greater than the first. A voltage gradient is then applied along the length of the microscale channel whereby the first, second and third fluid regions are transported along the length of the microscale channel with a first electroosmotic mobility, and whereby the material in the first fluid has an electrophoretic mobility different from the electroosmotic mobility, resulting in a concentration of the subject material at or near one end of the first fluid region.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic illustration of an embodiment of a microfluidic device for performing continuous serial dilution of a subject material.

Figure 2 is a schematic illustration of an alternate embodiment of a microfluidic device for performing continuous serial dilution of a subject material.

Figure 3 is a schematic illustration of an embodiment of a passive dilution module incorporated into a microfluidic device according to the present invention.

Figure 4 is a schematic illustration of a further embodiment of a microfluidic device for performing serial dilutions of a subject material in a stepwise format.

Figures 5A through 5E show a schematic illustration of a concentrator element for incorporation into a microfluidic system. Figure 5A illustrates the structure of the element, whereas Figures 5B through 5E illustrate the operation of the concentrator element in concentrating a particular subject material.

Figure 6 shows a channel and reservoir layout of a microfluidic device used to perform the dilution methods described herein.

Figure 7 shows two plots (Figures 7A and 7B) of fluorescence levels as a function of expected dye concentrations at various diliutions carried out according to the methods described herein. Figure 7A is a standard plot of fluorescent intensity versus expected dye concentration while Figure 7B shows the same plot in log scale for both axes.

DETAILED DESCRIPTION OF THE INVENTION

I. Generally

The present invention is generally directed to methods, apparatus and systems for use in performing *in situ* dilution or concentration of a particular subject material in a microfluidic device or system. These methods and apparatus may generally be

integrated with other microfluidic operations and/or systems, to perform a number of different manipulations, wherein dilution or concentration, carried out within the context of the microfluidic device or system, is just one part of the overall operation.

The term "dilution," as used herein, generally encompasses the ordinary meaning of that term, namely, the reduction in the amount of a particular subject material per unit volume of a fluid containing that material, through the addition of a second fluid, or diluent, to a first fluid which contains the subject material, e.g., soluble chemical component, or a suspension or emulsion of a partially insoluble material, whereby the resulting concentration of the subject material is reduced over that of the first fluid. In terms of the present invention, the diluent may take on a variety of forms, including aqueous or nonaqueous fluids and/or it may include additional material components, e.g., soluble chemical components or suspensions or emulsions of at least partially insoluble components. As alluded to above, the subject material may comprise virtually any composition, including chemical compounds, either soluble or as suspensions or emulsions, biological material, either soluble or as suspensions (e.g., cells) or emulsions, and the like. By "serial dilution" is generally meant successive dilutions, as defined herein, wherein the subject material is diluted with diluent to form a first diluted material, which first diluted material is then diluted with a diluent again, to produce a second diluted material, etc. For example, one produces a first diluted material that is diluted 1:10 over the subject material. By then diluting at least a portion of this material 1:10, one produces a second diluted material that is a 1:100 dilution of the subject material. In general, the methods, devices and systems of the present invention are useful in diluting subject material greater than 10 fold (1:10), typically greater than 100 fold (1:100), preferably greater than 1000 fold (1:1000), and in many cases, greater than 10,000 fold (1:10,000), within a single integrated microfluidic device, which typically has an internal volume, e.g., channel volume, of less than 10 µl and preferably less than 1 µl.

The term "concentration" as used herein, generally refers to the ordinary meaning of that term, namely the increase in the amount of a particular subject material per unit volume of the fluid in which the material is disposed, e.g., wholly or partially dissolved, suspended, slurried, etc.

As used herein, the term "microfluidic," or the term "microscale" when used to describe a fluidic element, such as a passage, chamber or conduit, generally refers to one or more fluid passages, chambers or conduits which have at least one internal cross-

sectional dimension, e.g., depth or width, of between about 0.1 μm and 500 μm. In the devices of the present invention, the microscale channels preferably have at least one cross-sectional dimension between about 0.1 μm and 200 μm, more preferably between about 0.1 μm and 100 μm, and often between about 0.1 μm and 20 μm. Accordingly, the microfluidic devices or systems of the present invention typically include at least one microscale channel, and preferably at least two intersecting microscale channels disposed within a single body structure.

The body structure may comprise a single component, or an aggregation of separate parts, e.g., capillaries, joints, chambers, layers, etc., which when appropriately mated or joined together, form the microfluidic device of the invention, e.g., containing the channels and/or chambers described herein. Typically, the microfluidic devices described herein will comprise a top portion, a bottom portion, and an interior portion, wherein the interior portion substantially defines the channels and chambers of the device. In preferred aspects, the bottom portion will comprise a solid substrate that is substantially planar in structure, and which has at least one substantially flat upper surface. A variety of substrate materials may be employed as the bottom portion. Typically, because the devices are microfabricated, substrate materials will generally be selected based upon their compatibility with known microfabrication techniques, e.g., photolithography, wet chemical etching, laser ablation, air abrasion techniques, injection molding, embossing, and other techniques. The substrate materials are also generally selected for their compatibility with the full range of conditions to which the microfluidic devices may be exposed, including extremes of pH, temperature, salt concentration, and application of electric fields. Accordingly, in some preferred aspects, the substrate material may include materials normally associated with the semiconductor industry in which such microfabrication techniques are regularly employed, including, e.g., silica based substrates such as glass, quartz, silicon or polysilicon, as well as other substrate materials, such as gallium arsenide and the like. In the case of semiconductive materials, it will often be desirable to provide an insulating coating or layer, e.g., silicon oxide, over the substrate material, particularly where electric fields are to be applied.

In additional preferred aspects, the substrate materials will comprise polymeric materials, e.g., plastics, such as polymethylmethacrylate (PMMA), polycarbonate, polytetrafluoroethylene (TEFLON™), polyvinylchloride (PVC), polydimethylsiloxane (PDMS), polysulfone, and the like. Such substrates are readily

manufactured from microfabricated masters, using well known molding techniques, such as injection molding, embossing or stamping, or by polymerizing the polymeric precursor material within or against the mold or master. Such polymeric substrate materials are preferred for their ease of manufacture, low cost and disposability, as well as their general inertness to most extreme reaction conditions. Again, these polymeric materials may include treated surfaces, e.g., derivatized or coated surfaces, to enhance their utility in the microfluidic system, e.g., provide enhanced fluid direction.

The channels and/or chambers of the microfluidic devices are typically fabricated into the upper surface of the substrate, or bottom portion, using the above described microfabrication techniques, as microscale grooves or indentations. The lower surface of the top portion of the microfluidic device, which top portion typically comprises a second planar substrate, is then overlaid upon and bonded to the surface of the bottom substrate, sealing the channels and/or chambers (the interior portion) of the device at the interface of these two components. Bonding of the top portion to the bottom portion may be carried out using a variety of known methods, depending upon the nature of the substrate material. For example, in the case of glass substrates, thermal bonding techniques may be used which employ elevated temperatures and pressure to bond the top portion of the device to the bottom portion. Polymeric substrates may be bonded using similar techniques, except that the temperatures used are generally lower to prevent excessive melting of the substrate material. Alternative methods may also be used to bond polymeric parts of the device together, including acoustic welding techniques, or the use of adhesives, e.g., UV curable adhesives, and the like.

The microfluidic devices and systems of the present invention, e.g., which are capable of performing *in situ* dilution or concentration of a particular subject material, typically comprise a main microscale channel disposed within the body structure, which is used to transport, and in some embodiments, mix the subject material and the diluent. Accordingly, the devices of the present invention comprise at least a first source of fluid containing a particular subject material in fluid communication with the main channel. For those aspects of the present invention directed to dilution of the subject material, a source of diluent is also generally provided in fluid communication with the main channel. Typically, the source of material and the source of diluent are provided as an integral part of the body structure, e.g., as wells or reservoirs within the body structure, e.g., the top portion of the device. Fluid communication between these sources and the main channel is generally via a

connecting channel which is in fluid communication with the well or reservoir at one end, and in fluid communication with, e.g., intersecting, the main channel at the other end.

In order to manipulate materials within the microfluidic devices described herein, the overall microfluidic systems of the present invention typically include a material direction system to manipulate selected materials within the various channels and/or chambers of the microfluidic device. Thus, by "material direction system" is meant a system which controls the movement and direction of fluids containing such materials within intersecting channel structures of a microfluidic device. Generally, such material direction systems employ pumps or pressure systems, and valves to affect fluid movement and direction in intersecting channels. A large number of microfabricated mechanical pumps and valves have been previously described in the art. Although such fluid direction elements may be useful in many aspects of the present invention, by and large, these elements are not preferred due to the complexity and cost of their manufacture. Further, the limits of microfabrication technology with respect to such pumps and valves, do not readily permit the manufacture of such elements that are capable of precisely handling sufficiently small volumes, e.g., volumes less than 1 µl. Thus, in particularly preferred aspects, the microfluidic systems of the present invention employ electroosmotic material direction systems to affect direction and transport of fluid borne materials within the microfluidic devices and systems of the invention. "Electroosmotic material direction systems," as used herein, refer to material direction systems which employ controlled electroosmotic flow to affect fluid movement and direction in intersecting channel structures. In particular, such systems function by applying a voltage gradient across the length of a fluid filled channel, the surface or walls of which have charged or ionizeable functional groups associated therewith, to produce electroosmotic flow of that fluid within that channel. Further, by concurrently regulating flow in two or more channels that meet at an intersection, one can direct fluid flow at that intersection. Such electroosmotic material direction systems and controllers are described in detail in, e.g., Published PCT Application No. 96/04547 to Ramsey et al., U.S. Application No. 08/691,632, filed August 2, 1996 and U.S. Application No. 08/761,986, filed December 6, 1996, each of which is incorporated herein by reference in its entirety for all purposes.

II. Dilution

As described above, in a first aspect, the present invention generally provides methods, devices and systems for performing the *in situ* dilution, and particularly serial dilution, of a subject material within a microfluidic device or system.

Dilution of the subject material in microfluidic devices and systems according to the present invention, generally comprises transporting a first volume of fluid containing the subject material from the reservoir containing that subject material, into the main channel, and mixing that first volume with a second volume of diluent transported into the main channel from the reservoir of diluent, to produce a first diluted material. The first diluted material is then further diluted by combining a portion of the first diluted material with another volume of diluent to produce a second diluted material. These dilution steps may be repeated any number of times until the desired dilution is achieved.

In preferred aspects, the process of mixing the fluid containing the subject material and the diluent preferably is carried out within the main channel. In particular, the first volume of the fluid containing the subject material is delivered from the source of the subject material into the main channel, while a second volume of diluent is transported from the diluent source into the main channel. The mixing of these two fluids within the main channel produces the first diluted material. This first diluted material is then combined with a further volume of diluent to produce a second diluted material, and so on.

However, because volumes of microfluidic devices and/or systems are extremely small and therefore limited, in order to further dilute the first diluted material, e.g., in a serial dilution, one must transport at least a portion of the first diluted material out of the main channel, e.g., out of the device or into a waste reservoir, prior to delivering the further volume of diluent, in order to accommodate that additional volume of diluent. Once the first diluted material is transported out of the main channel, a further volume of diluent is delivered to the main channel and combined with the remaining portion of the first diluted material, which, upon mixing, produces a second diluted material which is the product of the first and second dilutions, e.g., if the first and second dilutions were each 10 fold, then the second diluted material would be a 100 fold dilution of the starting material.

One example of a microfluidic device for carrying out *in situ* serial dilution as described herein, is schematically illustrated in Figure 1. As shown, the device 100, includes a body structure 102 which has a main channel 104 disposed therein. As shown, a source of, or reservoir containing the subject material 106 is provided in fluid

communication with the main channel. The illustrated device also includes multiple diluent sources 110, 114 and 118 in fluid communication with the main channel via diluent channels 122, 126 and 130, respectively. The device also includes reservoirs 112, and 116 in fluid communication with the main channel via reservoir channels 124, 128 and 132, respectively. The main channel is also shown with a terminal waste reservoir 108 at its terminus. Although illustrated in terms of performing only dilutions of the subject material, it will be appreciated from the instant disclosure that this device or aspect of a device is readily integrated into a device or system which performs numerous other manipulations, including enzyme assays, immunoassays, screening assays, separations and the like. As a result, waste reservoir 108 may generally be substituted with appropriate fluidic elements for performing further manipulations of the ultimately diluted material, e.g., analysis, reaction, detection, etc.

In operation, a first volume of the subject material from reservoir 106 is flowed into the main channel 104. This volume of subject material is combined with a first volume of diluent which is flowed into main channel 104 from diluent reservoir 110, via channel 122. While flowing down the main channel towards reservoir 108, the subject material and the diluent will mix to form a first diluted material. A portion of this first material is then directed, or tapped off, from the first channel into reservoir channel 124 and out to reservoir 112, while the remaining portion of first diluted material continues to flow down the length of the main channel towards reservoir 108. This remaining portion of first diluted material is then combined with a second volume of diluent delivered into main channel 104 from diluent reservoir 114 via channel 126, whereupon, the second volume of diluent and first diluted material will mix to form a second diluted material flowing down the main channel towards reservoir 108. For ease of discussion, each step of directing a portion of the diluted material out of the main channel and delivering a volume of diluent into the channel to combine with the remaining portion of diluted material is referred to herein as a dilution stage. A portion of this second diluted material is then directed from the first channel into reservoir channel 128 and out to reservoir 116, while the remaining portion of second diluted material continues to flow down the main channel towards reservoir 108. The remaining portion of the second diluted material is then combined with a third volume of diluent delivered into main channel 104 from diluent reservoir 118 via channel 130, whereupon the third volume of diluent and the remaining portion of the second diluted material will mix to form a third diluted material. This third diluted material may

then be subjected to a subsequent analysis, reaction, combination etc. However, in preferred aspects, and as shown in Figure 1, a portion of the third diluted material in main channel 104 is transported out of the main channel and into reservoir 120, via channel 132. This allows the stepping down of the volume of third diluted material which must be subsequently handled.

The steps of delivering diluent to the main channel and transporting portions of diluted material out of the main channel may be repeated as necessary in order to produce the dilution ratio desired. In addition, although described in terms of volumes of material and diluent, the devices of the present invention as they relate to dilution, preferably are run in a continuous flow arrangement. Specifically, subject material is flowed into the main channel in a stream at a first flow rate, while a stream of diluent is concurrently flowed into the main channel at a second flow rate, where the ratio of first flow rate to second flow rate are related to the desired dilution for that particular dilution step. These streams mix within the main channel to form a stream of first diluted material. Similarly, a portion of the stream of first diluted material is continuously flowed out of the main channel into the reservoir channel and out to the reservoir, while downstream, another stream of diluent is introduced into the main channel to mix with the remaining portion of the stream of first diluted material to produce second diluted material. These steps are repeated on down the main channel to form a continuous stream of material at the desired dilution as long as flow is maintained into and out of the main channel at the appropriate rates to affect the incremental dilutions at each dilution stage.

The dilution methods and systems described herein are particularly useful in microfluidic systems which employ electroosmotic fluid direction systems. In these electroosmotic fluid direction systems, fluid flow is generally proportional to current flow. Accordingly, in performing dilutions using these systems, the dilution ratio is generally related to the ratio of current applied at the subject material reservoir to the current applied to the diluent reservoir. For relatively small dilutions, e.g., 1:10, these electroosmotic systems can carry out the desired dilution in one step, e.g., combining 1 part subject material to 9 parts diluent, or applying 1 μA to the subject material reservoir and 9 μA to the diluent reservoir. However, for dilutions somewhat greater than 1:10, diffusional leakage of the subject material from its supply channel into the main channel limits further dilution, even where only extremely small currents are applied at the subject material reservoir.

One potential solution to the problem of diffusional leakage is to perform multiple serial dilutions, e.g., perform three successive 10 fold dilutions. However, because fluid flow is related to current flow, the final dilution would require a current applied at the dilution well of 900 μA. Specifically, the first dilution would be carried out by applying 1 μA to the subject material reservoir while applying 9 μA to the diluent reservoir, resulting in a 10 μA current in the main channel. The second 1:10 dilution would then require a current of 90 μA in the second diluent reservoir, and create a current of 100 μA in the main channel. The final dilution would then require an applied current at the third diluent reservoir of 900 μA, which would create a large number of adverse effects on the microfluidic system, electrolysis of the buffers, excessive heating of the fluid channels, etc.

As described above, the methods and systems of the present invention combine the subject material with diluent to create a first diluted material. A portion of this diluted material is then removed from the main channel and the remaining portion is further diluted. This process allows one to perform serial dilutions as described above, the methods and systems of the present invention combine the subject material with diluent microfluidic systems, thereby obviating the problem of diffusional leakage at the intersections, as well as permitting much larger dilutions. Furthermore, this process allows for the use of electroosmotic fluid direction systems without requiring excessively large current ratios, and thus, application of excessive currents, which can produce potentially undesirable joule heating within the microchannels.

In this respect, the methods and devices of the invention are used to maintain current levels within microfluidic channels below excessive levels, despite the need to apply multiple currents to those channels, e.g., to introduce additional materials to the system. For example, in a microfluidic device in which first and second currents are applied to a first fluid region, e.g., a channel, channels chamber or chambers, the total of the first and second applied currents often can exceed a desired maximum total current. Thus, the present invention provides methods of maintaining a current in the first fluid region below the desired maximum total current, by applying at least a third current to the first fluid region that is sufficient to reduce the current in the first fluid region to below the desired maximum current. As described in different portions of this disclosure, this is often referred to as "tapping off" the current.

Typically, the third current is applied at a point in the first fluid region that is between a point in the first fluid region at which the first current is applied and a point in

the first fluid region at which the second current is applied, so as to tap off excessive current before the additional current is added. In many aspects, the third current is substantially opposite to at least one of the first and second currents. For example, in dilution embodiments an amount of current is typically tapped off that is substantially equal to the amount of current that is to be added in the next dilution step. In the case of the methods described herein, the first current often comprises an aggregate of fourth and fifth currents, which were applied to the system to cause cause movement of a first material and a first diluent into the first fluid region, e.g., by applying the currents to a material reservoir and diluent reservoir fluidly connected to the first fluid region. Application of these currents to the material and diluent reservoirs then causes electrokinetic movement (electrophoretic or electroosmotic).

In accordance with the methods described, the third current is typically applied to a first waste reservoir that is in fluid communication with the first fluid region. In many aspects, application of the third current causes movement of at least a portion of the first diluted material from the first fluid region into the first waste reservoir.

In order to avoid adverse effects of excess currents applied to microfluidic channels, it is generally desirable to maintain the current within the microscale channel at a level that is less than about 10 mA, typically less than about 1 mA, preferably less than about 100 μA, more preferably less than or equal to about 20 μA, and often less than or equal to about 10 μA.

Referring to the system illustrated in Figure 1, a particular subject material disposed in reservoir 106, is diluted up to 1,000 fold using these methods. In particular, a first 10 fold dilution of the subject material is achieved by applying 1 μA to reservoir 106, while applying 9 μA to the first diluent reservoir 110, resulting in a 9 to 1 ratio of diluent flow to subject material flow. This also results in a 10 μA current within the main channel 104, between its intersection with channel 122 and its intersection with channel 124. A current of -9 μA is applied at waste reservoir 112, which results in a tapping off of 90 % of the current and flowing of approximately 90 % of the diluted material into the waste reservoir from the main channel, and returns current in the main channel to 1 μA between its intersection with channel 124 and its intersection with channel 126. Again, a 9 μA current is applied in second diluent reservoir 114, resulting in a further 1:10 dilution of the subject material within the main channel, or a total dilution of 1:100. Another -9 μA current is then applied at waste reservoir 116, withdrawing approximately 90 % of the

second diluted material, and the final dilution step is supplied by applying 9 μA at third diluent reservoir 118. A further waste reservoir 120 is also shown, for reducing the current at the final waste reservoir 108, back to 1 μA. Accordingly, a 1000 fold dilution only requires a 10 fold current range across the device. Further, because each dilution step is relatively modest, e.g., 1:10, these methods do not suffer from the problems of diffusional leakage at the combining intersection, because the smallest volume is easily controlled at these levels.

An alternative structure that can be used in practicing the dilution methods described herein, is shown in Figure 2. This alternate structure has the advantage of including a potentially unlimited number of dilution stages while only requiring six reservoirs at which electrical contact is made. As shown, the channel structure is shown fabricated in a substrate 102 and again includes subject material reservoir 106, main channel 104 and waste reservoir 108. In addition, the structure includes a main diluent channel 204 which has at its termini diluent reservoirs 202 and 206, and main waste channel 210 which has at its termini waste reservoirs 208 and 212. Main diluent channel 204 is in fluid communication with main channel 104 via a plurality of diluent connecting channels 214, 216 and 218. Similarly, main waste channel 210 is in fluid communication with main channel 104 via waste connecting channels 220, 222 and 224, which are staggered from the diluent connecting channels, e.g., diluent connecting channel 214 intersects main channel 104 at a first point, and waste connecting channel 220 intersects the main channel 204 at a second point, that is further downstream from the first point.

In operation, subject material from reservoir 106 is introduced into main channel 104, e.g., by applying a current along the length of channel 104. A first volume of diluent is introduced into the main channel from main diluent channel 204, via diluent connecting channel 214, to create a first diluted material, which continues to flow toward reservoir 108. After the subject material and diluent mix, a first portion of the first diluted material is transported out of main channel 104 into main waste channel 210, via waste connecting channel 220. A second volume of diluent is then delivered to the main channel 104 from main diluent channel 204, via diluent connecting channel 216, whereupon it will mix with the remaining portion of first diluted material to form a second diluted material. A portion of this second diluted material is then transported out of channel 104 into the main waste channel 210 via waste connecting channel 222. The process steps are then repeated, e.g., with diluent connecting channel 218 and waste connecting channel 224, as necessary to

achieve the desired dilution. As shown, the channel structure includes three dilution stages. However, as noted previously, the structure shown can include additional dilution stages without necessitating additional reservoirs. For example, in the figure shown, application of a first voltage to reservoirs 106, 202 and 208, and a second voltage to reservoirs 108, 206 and 212 will cause material to move in parallel down each of main diluent channel 204, main channel 104, and main waste channel 210, with no dilution of the subject material in main channel 104. Specifically, no material will move among the various main channels. Increasing the potentials at reservoirs 202 and 206, while symmetrically reducing the potentials applied at reservoirs 208 and 212, relative to the potentials applied at reservoirs 106 and 108, will cause dilution to occur in the main channel 104. Specifically, application of these voltage gradients will cause diluent to flow from main diluent channel 204 through diluent connecting channels 214, 216 and 218, diluting the subject material present in the main channel at the intersection of the main channel and each of the diluent connecting channels. Similarly, material in the main channel, in addition to flowing toward reservoir 108, will also be flowing out of main channel 104 via waste connecting channels 220, 222 and 224 and thereby creating room for incoming diluent in a subsequent stage.

In addition to the foregoing, it will be appreciated from the instant disclosure, that the length of the diluent and waste channels in Figures 1 and 2 may be adjusted, in order to obviate the need for a wide range of applied voltages to achieve the desired currents. Specifically, higher voltages are required to achieve a certain desired current in a channel with higher resistance. By reducing the length of the channel, one also reduces the resistance along the length of that channel.

As will be apparent from the disclosure of the invention herein, in many of the embodiments described, either the flow rates of the materials and diluents, or the length of the main channel between the diluent channels and the waste channels are selected whereby the subject material and the diluent are permitted to adequately mix before a portion of these materials is transported out of the main channel. These parameters will typically vary depending upon the diffusion rate for the material being transported, the length of the channel along which the material is being transported, and the flow velocity of the material being transported. Generally, however, these parameters are readily determinable with only routine experimentation, and may be readily optimized depending upon the overall system used.

Both structures illustrated in Figures 1 and 2, provide for dilution of the subject material based upon the currents or voltages applied to the device, as well as the number of dilution modules or stages fabricated into the device. In a further modified aspect, the present invention also provides a dilution module that yields a given dilution of the subject material regardless of the current applied through the system, effectively 'hard-wiring' a particular dilution factor into the device.

A schematic illustration of one example of such a dilution module is shown in Figure 3. As shown in Figure 3, the device again includes a main channel 104, which includes a narrowed region 302. Bypass channel 304 fluidly connects with main channel 104 above and below narrow region 302, i.e., upstream and downstream of the narrow region, and includes a diluent reservoir 306, disposed therein. In particular, diluent reservoir 306 is an integral part of the bypass channel 304. Reservoir 306 typically has a volume that is sufficiently larger than the volume of the channels, such that regardless of the flow of subject material into the reservoir via bypass channel 304, the effluent from the reservoir has an insignificant concentration of subject material. Because of the extremely small volumes of material required in these microfluidic systems, effective volumes of this diluent reservoir can be on the order of 1 to 10 μl, and less, depending upon the flow rate used, the length of time the dilution is to run and the like. The increased volume of diluent reservoir 306 may be provided by increasing the length, width and/or depth of the reservoir relative to the channels of the device.

The size of the dilution accomplished by this type of dilution module is dictated by the ratio of the resistance of channel 302 to the resistance of the loop defined by channels 304 and reservoir 306, regardless of the level of current flowing through the dilution module as a whole. In particular, as noted above, in electroosmotic flow systems, fluid flow is proportional to the level of current flow along a channel, which is inversely proportional to the resistance along a given channel. The resistance through a given fluid filled channel is inversely proportional to the cross sectional area, and directly proportional to the length of the channel through which current is passed. Accordingly, by adjusting the resistance along two potential current paths in a fluidic system, one can control the amount of electroosmotic fluid flow along each of the channels. For example, if fluid channels A and B begin and terminate in fluid communication, one can flow approximately 90% of fluid along path A by providing 90% of the current along this path. This is done by increasing the resistance along path B relative to path A. One simple method of increasing

this resistance is to narrow the cross sectional dimensions of path B relative to path A. As can be appreciated, other methods may also be used to increase the resistance along path B, including increasing the length of path B relative to path A.

Thus, as can be readily appreciated from the above, and with reference to Figure 3, the ratio of subject material flowing out of main channel 104, and thus, the amount of diluent flowing into the main channel below the narrow region 302, to the amount of material flowing through the narrow region 302, is proportional to the ratio of the resistance of the narrow region to that of the bypass region. This is the case regardless of the amount of current flowing through the system. Dilution occurs at the lower end of the narrow region, where subject material flowing into reservoir 306 displaces an equal volume of diluent, which flows into the main channel at the lower end of the narrow region and mixes with the portion of subject material that traveled through the narrow region, to create a first diluted material. Because the diluent reservoir 306 has a sufficiently large volume, as compared with the amount of subject material flowing therein, the effluent is substantially entirely diluent. Rephrased, any amount of subject material coming through the reservoir is negligible when considered in light of the amount of subject material present in the first diluted material. Additional dilution modules can be included within a device, depending upon the level of dilution ultimately desired. Further, in those applications where one wishes to manipulate a number of different dilutions of a given subject material, e.g., in enzyme assays, binding assays, inhibitor screening assays, and the like, one can aliquot a sample of diluted material from the main channel after each dilution stage, for further manipulation, while the remainder of the material is subject to further dilution stages and manipulations.

Another closely related example of a microfluidic system for performing *in situ* dilutions is schematically illustrated in Figure 4. Again, the methods and devices described in this example perform serial dilutions of the subject material, and maintain the volume of material within the main channel by removing a portion of the diluted material from the channel following each step. This example differs from that described above, however, in that it includes a single diluent reservoir 402, but employs mixing chambers or regions, 406, 408 and 410, separate from the main channel 104, for mixing the diluent and subject material, in a number of steps. In particular, a first volume of the subject material is transported from reservoir 106 to reservoir 406, where it is mixed with a first volume of diluent transported to chamber 406 from reservoir 402, to form a first diluted material.

Typically, the transport of the subject material and the first volume of diluent is carried out concurrently, although this is not necessary. Typically, the mixing chambers will have a substantially larger cross sectional dimension and/or depth than the channels of the system, to allow the introduction of larger volumes. A second volume of the first diluted material, e.g., typically some fraction of the total volume of the first diluted material, is then transported from mixing chamber 406 to mixing chamber 408, via main channel 104, whereupon it is mixed with a second volume of diluent to create a second diluted material. The second volume of first diluted material and second volume of diluent are selected depending upon the dilution that is desired for this step. Again, the transportation of the first diluted material and the second volume of diluent to mixing chamber 408 is typically carried out concurrently. This step is repeated in mixing chamber 410 and further as needed for the desired ultimate dilution.

The present invention also provides for the use of a microfluidic device for performing *in situ* dilution of a first material, the device comprising a first dilutiuon region in fluid communication with a source of the material, first and second diluent sources in fluid commnication with the dilution region, and a fluid transport system for selectively delivering the first material and first diluent to the first dilution region to produce a first diluted material, for transporting a portion of the first diluted material out of the first dilution region and for transporting the second diluent to the first dilution regoion to create a second diluted material.

III. Concentration

In another aspect, the present invention is directed to methods of performing in situ concentration of samples within microfluidic devices and systems, as well as being directed to the devices and systems themselves. In particular, the devices and systems concentrate a particular subject material electrophoretically, within the channels of the microfluidic device, and are therefore particularly suited for use with electroosmotic fluid direction systems, e.g., as described above. Specifically, in electroosmotic fluid direction, the presence of charged groups within a particular subject material or chemical specie can result in the electrophoretic mobility of that material within the system being different from the electroosmotic mobility of the fluid carrying that material. Depending upon the charge, this may result in the subject material moving through the channels of the system faster or slower than the fluid in which it is disposed. This shifting or electrophoretic biasing of the

subject material within its fluid carrier can result in problems where one's goal is to transport the subject material from a first location in the microfluidic system to a second location in the system, substantially unchanged. In particular, these electroosmotic fluid direction systems function by applying a voltage gradient or electric field across the length of a channel along which fluid flow is desired. This same voltage gradient or electric field also causes the charged species within the fluid contained in the channel to electrophoreses. Where the voltage gradient is greater, e.g., in lower conductivity fluids, the electrophoresis of the charged species is also greater, while lower voltage gradients, e.g., in high conductivity fluids, result in lower levels of electrophoresis.

The present invention employs both low and high conductivity fluids in the same channel, to affect the concentration of the subject material. Specifically, the subject material is generally provided in a fluid or buffer having a low relative conductivity, and dispensed as a discrete volume or fluid region, into a microscale channel, surrounded by fluid regions (also termed "spacer regions") of high relative conductivity. The fluid of high relative conductivity will typically have a conductivity that is at least two times the conductivity of the low relative conductivity fluid, and preferably, at least five times the conductivity of the low relative conductivity fluid, more preferably at least ten times the conductivity of the low relative conductivity fluid, and often at least twenty times the conductivity of the low relative conductivity fluid. Typically, the low conductivity fluid will have a conductivity in the range of from about 0.1 mS to about 10 mS. The high conductivity fluid typically has a conductivity in the range of from about 0.2 mS to about 100 mS.

When a voltage gradient is applied across the length of the channel, it will result in the movement of the subject material fluid along the channel by electroosmotic flow. The voltage gradient will also result in the electrophoresis of the charged subject material in the same or opposite direction of electroosmotic flow within the channel. The direction of electrophoretic mobility is determined by the charge on the subject material, e.g., either positive or negative. However, once the charged subject material reaches the interface of the low conductivity subject material region and the high conductivity spacer region, the electrophoresis of the charged material will be substantially reduced, because of the lack of a substantial voltage gradient across the high conductivity regions. This results in a "stacking" or concentration of the charged species at or near the interface of the two fluids. A portion of the fluid at or near this interface is then aliquoted from the remainder

of the fluid in the channel at a substantially concentrated level, for further processing or analysis, e.g., separation.

An example of a concentrator device, or concentrator elements of a device, for performing this type of concentration is schematically illustrated in Figure 5. As shown in Figure 5A, the concentrator includes a main channel 502, a sample introduction channel 504 in fluid communication with the main channel at a first intersection 506, a waste channel 508 in fluid communication with the main channel at a second intersection 510, and an aliquoting channel 512 in fluid communication with the main channel at a third intersection 514. The aliquoting channel may be used to shunt off the concentrated portion of the subject material. Alternatively, the stacked subject material may merely be subject to further manipulation as is. For example, in screening applications, e.g., employing inhibition assays, binding assays or the like, the concentration of the screened compound at the front of the fluid material that is loaded into the main channel will often be sufficient to ascertain screen results. Similarly, the concentration of species at the front edge of the material region may also act as a stacking process, e.g., like a stacking gel layer in SDS-PAGE or agarose sequencing gels, in order to provide greater resolution in separation applications, e.g., capillary electrophoresis.

As shown the third intersection 514 is provided in the main channel somewhat dislocated from the first and second intersections 506 and 510, respectively, to provide adequate opportunity for the electrophoresis of the charged materials. Again, this distance will generally vary depending upon the nature of the material sought to be concentrated, e.g., its net charge, molecular weight, conformation, or other factors affecting the materials electrophoretic mobility within the channel.

The concentrator illustrated in Figure 5A also includes shallow regions 516 and 518 within the sample loading and waste channels 504 and 508, respectively, where these channels reach their respective intersections with the main channel 502. These shallow regions generally prevent unwanted flow due to pressure effects associated with the electroosmotic pumping of different fluid regions within a microscale channel intersected by other channels, where those different fluid regions have different conductivities. This is described in substantial detail in U.S. Patent Application Serial No. 08/760,446, filed December 6, 1996, and incorporated herein by reference in its entirety for all purposes.

Briefly, in the electroosmotic flow systems described herein, the presence of differentially mobile fluids (e.g., fluids having different conductivities and thus having

different electroosmotic or electrokinetic mobilities in the particular system) in a channel may result in multiple different pressures being present along the length of a channel in the system. For example, these electrokinetic flow systems typically employ a series of regions of low and high conductivity fluids (e.g., high ionic concentration spacer regions and low ionic concentration material regions) in a given channel to produce electroosmotic flow. As the low ionic concentration regions within the channel tend to drop the most applied voltage across their length, they will tend to push the fluids through a channel. Conversely, high conductivity fluid regions within the channel provide relatively little voltage drop across their lengths, and tend to slow down fluid flow due to viscous drag.

As a result of these pushing and dragging effects, pressure variations can generally be produced along the length of a fluid filled channel. The highest pressure is typically found at the front or leading edge of the low ionic concentration regions (e.g., the material regions), while the lowest pressure is typically found at the trailing or back edge of these low ionic concentration fluid regions.

By reducing the depth of the channels intersecting the main channel, e.g., the sample introduction and waste channels 516 and 518, relative to the main channel 502, the fluctuations in fluid flow can be substantially eliminated. In particular, in electroosmotic fluid propulsion or direction, for a given voltage gradient, the rate of flow (volume/time) generally varies with the depth of the channel for channels having an aspect ratio of >10 (width:depth). With some minor, inconsequential error for the calculation, this general ratio also holds true for lower aspect ratios, e.g., aspect ratios >5. Conversely, the pressure induced flow for the same channel will vary as the third power of the channel depth. Thus, the pressure build-up in a channel due to the simultaneous presence of fluid regions of differing ionic strength will vary as the square of the channel depth.

Accordingly, by decreasing the depth of the intersecting second channel relative to the depth of the first or main channel by a factor of X, one can significantly reduce the pressure induced flow, e.g., by a factor of X^3, while only slightly reducing the electroosmotically induced flow into that channel, e.g., by a factor of X. For example, where the second channel is reduced in depth relative to the first channel by one order of magnitude, the pressure induced flow into that second channel will be reduced 1000 times while the electroosmotically induced flow will be reduced by only a factor of ten. Accordingly, in some aspects, the present invention provides microfluidic devices as generally described herein, e.g., having at least first and second intersecting channels disposed therein, but where the first channel is deeper than the second channel. Generally,

the depths of the channels may be varied to obtain optimal flow conditions for a desired application. As such, depending upon the application, the first channel may be greater than about two times as deep as the second channel, preferably greater than about 5 times as deep as the second channel, and often greater than about ten times as deep as the second channel.

The operation of the concentrator element is schematically illustrated in Figure 5B through 5E. As shown, the sample fluid containing the subject material and having a low conductivity (shown in grey) is loaded into the main channel by transporting the material from a sample channel 504 to waste channel 508 via the main channel 502, typically by applying a voltage gradient along this path. The direction of fluid flow is indicated by the arrows. As noted above, the sample fluid in the main channel (shaded region) has a low relative conductivity, and is typically bounded by fluid regions having relatively high conductivity (shown unshaded). Transport of the sample fluid into the main channel is typically carried out by application of a voltage gradient between reservoirs that are separately in fluid communication with the sample and waste channels. Switching the voltage gradient across the length of main channel 502, i.e., by applying different voltages at the termini of the main channel, then allows the transport of the subject material along that channel (Figure 5C). Concurrent with this transport is the electrophoretic stacking of the charged subject material within the subject material fluid, indicated by the darker shading at the front of the sample fluid plug being transported along the main channel (Figures 5C and 5D). Although illustrated in terms of the subject material being concentrated at the front of the subject material region, it will be readily appreciated that the charge on the subject material will affect the mobility of that material under an electric field. For example, if the subject material possesses a net charge opposite to that for which concentration is illustrated in Figure 5B-5D, then the subject material will be concentrated at the back end of the subject material region, relative to the direction of electroosmotic flow.

Finally, as shown in Figure 5E, when the concentrated or stacked portion of the sample fluid reaches the third intersection, the concentrated portion can be shunted off from the remainder of the sample fluid plug by appropriate application of a voltage gradient along that path, effectively resulting in the concentration the subject material. In some cases, it is not necessary to shunt off the concentrated material in order to see the benefit of the concentration of the material. For example, where screening compounds of relatively low concentration, the increased localized concentration of the compound at the front end of

the low ionic concentration region resulting from the concentration methods described herein may be sufficient to produce an adequately detectable result.

The present invention provides for the use of any of the devices or systems described herein for either in situ dilution or concentration of materials within a microscale fluidic channel.

EXAMPLES

The following example illustrates the use of the above described methods and apparatuses in performing in situ, e.g., on-chip, dilutions in microfluidic channels. A microfluidic device having the channel/reservoir layout shown in Figure 6 was used to perform up to 1000-fold dilutions with applied currents that ranged between -10 and +10μA.

A reagent, in this example the fluorescent dye rhodamine, was placed in reservoir 8 of the device and diluent (200mM CAPS buffer) was placed in all other reservoirs (1-7). Electrical currents were applied to the fluid wells to electrokinetically pump the fluids towards reservoir 4, the waste reservoir. The fluorescent intensity in the channel was measured by a PMT at the detection point near the waste well. Plots of the fluorescent intensity for the various dilution states is shown in Figures 7A (fluorescent intensity vs. expected dye concentration) and 7B (log scale). The undiluted dye was first measured by applying 10μA to reservoir 8 and 0 μA to reservoir 1, to transport straight dye along the main channel past the detection point. Dilutions that included from 90% down to10% of the original dye level, at 10% increments were created by varying the currents at channels 8 and 1 as shown in states 1-10 in Table 1, below. Dye levels from 9% down to 1%, at 1% increments, were created by tapping out some of the dye mixture created by currents applied to reservoirs 1 and 8 into waste reservoir 2 and replacing it with buffer from reservoir 7. These are states 11-19 in Table 1, below. Dilutions of 0.9 down to 0.1% of the original dye level, at 0.1% increments were created in a similar manner using reservoirs 3 (tapping off material) and 6 (adding more diluent). The expected dye level (as a fraction of original dye) is given by the following equation where I_n is the current at reservoir n. It assumes that the currents at channels 2 and 7 are equal and opposite. The same assumption was made on reservoirs 3 and 6.

$$DyeLevel = \left(\frac{I_8}{I_1 + I_8}\right)\left(\frac{I_1 + I_8 - I_7}{I_1 + I_8}\right)\left(\frac{I_1 + I_8 - I_6}{I_1 + I_8}\right)$$

In all states, 500V was applied at reservoir 4.

The extra path length introduced by the three curved sections in the main channel of the device design ensures that the dye and buffer in the channel were thoroughly mixed before any portion is tapped off. Mixing in the main channel occurs by diffusion so allowing extra time allows for more thorough mixing.

TABLE 1

State Number	Expected Dye Level	Applied Current Res. 1	Applied Current Res. 8	Applied Current Res. 2	Applied Current Res. 7	Applied Current Res. 3	Applied Current Res. 8
1	1	0	10	0	0	0	0
2	0.9	1	9	0	0	0	0
3	0.8	2	8	0	0	0	0
4	0.7	3	7	0	0	0	0
5	0.6	4	6	0	0	0	0
6	0.5	5	5	0	0	0	0
7	0.4	6	4	0	0	0	0
8	0.3	7	3	0	0	0	0
9	0.2	8	2	0	0	0	0
10	0.1	9	1	0	0	0	0
11	0.09	9	1	-1	1	0	0
12	0.08	9	1	-2	2	0	0
13	0.07	9	1	-3	3	0	0
14	0.06	9	1	-4	4	0	0
15	0.05	9	1	-5	5	0	0
16	0.04	9	1	-6	6	0	0
17	0.03	9	1	-7	7	0	0
18	0.02	9	1	-8	8	0	0
19	0.01	9	1	-9	9	0	0
20	0.009	9	1	-9	9	-1	1
21	0.008	9	1	-9	9	-2	2
22	0.007	9	1	-9	9	-3	3
23	0.006	9	1	-9	9	-4	4
24	0.005	9	1	-9	9	-5	5
25	0.004	9	1	-9	9	-6	6
26	0.003	9	1	-9	9	-7	7
27	0.002	9	1	-9	9	-8	8
28	0.001	9	1	-9	9	-9	9

The data from this experiment is shown in Figures 7A and 7B. In both Figures 7A and 7B, the y-axis denotes the net intensity of the fluorescent dye as read by the

PMT, with the background buffer reading subtracted. The x-axis provides the expected dye concentration as calculated by the above equation using the measured applied currents. The plot in Figure 7A shows a linear plot while Figure 7B shows a log-log plot to better illustrate the linearity of the data at the lower dilutions. The two dashed lines on each graph define the window that contains data that is within 10% of the calculated dilution as defined from the highest dye concentration.

As can be seen from these plots, the methods described herein can be used to accurately create substantial dilutions within a microfluidic system, while applying a relatively small, and easily controlled dynamic current range, e.g., from -10 μA to 10 μA.

All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. In the case of described methods, the steps of such methods are optionally practiced in any order. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

We Claim:

1. A method for *in situ* dilution of a material in a microfluidic device, comprising:

combining a first volume of said material with a first volume of diluent in a first microscale channel to form a first diluted material;

transporting at least a first portion of said first diluted material out of said first channel; and

delivering a second volume of diluent from a second diluent source to said microscale channel and combining said second volume of diluent with a second portion of said first diluted material to form a second diluted material.

2. The method of claim 1, wherein said first volume of diluent is a first preselected volume of diluent.

3. The method of claim 1, wherein said first portion is transported out of said microscale channel by transporting said first portion to a first reservoir in fluid communication with said first microscale channel.

4. The method of claim 1, wherein said combining, transporting and delivering steps are carried out in continuous flow.

5. The method of claim 1, wherein said combining step further comprises continuously flowing said material from a source of said material, and said first diluent from a first diluent source, into said microscale channel to form a stream of first diluted material in said microscale channel, said transporting step comprises continuously flowing a portion of said stream of first diluted material out of said microscale channel and said delivering step comprises continuously flowing said second diluent into said microscale channel and combining it with said a second portion of said stream of first diluted material to form a stream of second diluted material.

6. The method of claim 3, wherein said combining step comprises applying a first electrical current to said source of material and a second electrical current to

said first diluent source, whereby an amount of said material and an amount of said diluent are transported into said main channel, a ratio of said first current to said second current being directly related to a dilution ratio for said first diluted material.

7. The method of claim 3, wherein said transporting step comprises applying a third current to said first reservoir, whereby a portion of said first diluted material is transported from said main channel to said first reservoir, and said delivering step comprises applying a fourth current to said second diluent source, said fourth current being substantially opposite to said third current, whereby an amount of second diluent is delivered to said microscale channel, said amount of second diluent being substantially equal to said amount of first diluted material transported from said microscale channel in said transporting step.

8. The method of claim 1, further comprising:

transporting at least a first portion of said second diluted material out of said first channel; and

delivering a third volume of diluent from a third diluent source to said microscale channel and combining said third volume of diluent with a second portion of said first diluted material to form a second diluted material.

9. The method of claim 8, wherein said third diluted material is greater than a 1:10 dilution of the first diluted material.

10. The method of claim 8, wherein said third diluted material is greater than a 1:50 dilution of the first diluted material.

11. The method of claim 8, wherein said third diluted material is greater than a 1:100 dilution of first diluted material.

12. The method of claim 1, wherein said third diluted material is greater than a 1:10 dilution of said second diluted material.

13. The method of claim 1, wherein said third diluted material is greater than a 1:50 dilution of said second diluted material.

14. A method for *in situ* dilution of a material in a microfluidic device, comprising:

combining a first volume of said material with a first volume of diluent in a first region of a microfluidic device to form a first diluted material;

transporting a first portion of said first diluted material into a second region of said microfluidic device; and

delivering a second volume of diluent from a second diluent source to said second region and combining said second volume of diluent with a second portion of said first diluted material to form a second diluted material.

15. The method of claim 14, wherein said first region comprises a first part of a main channel in said microfluidic device, and said second region comprises a second part of said main channel, and said step of transporting a first portion of said first diluted material into said second region comprises transporting a second portion of said first diluted material out of said main channel.

16. The method of claim 14, wherein said first region comprises a first mixing chamber disposed in a microfluidic device and said second region comprises a second mixing chamber disposed in said microfluidic device, each of said mixing chambers being in fluid communication with a microscale channel, said microscale channel being in fluid communication with a source of said material and a source of said diluent, and wherein said step of transporting a first portion of said first diluted material to said second region comprises transporting said first portion from said first mixing chamber to said second mixing chamber.

17. A method for performing *in situ* dilution of a material in a microfluidic device, comprising:

providing a microfluidic device that comprises disposed therein, a first dilution region, a first source of fluid material fluidly connected to the first dilution region, and first and second diluent sources fluidly connected to the first dilution region;

applying a first current between the first source of material and the first dilution region to transport an amount of the first material into the first dilution region;

applying a second current between a first source of diluent and the first dilution region that is in fluid communication with the first source of diluent to transport an amount of first diluent into the first dilution region, the amounts of first material and first diluent mixing to form a first diluted material, the first and second currents combining in the first dilution region to produce a third current therein;

tapping at least a portion of the third current from the first dilution region; and

applying a fourth current between the second diluent source and the first microscale channel to transport an amount of the second diluent to the first dilution region.

18. The method of claim 17, wherein the step of tapping at least a portion of the third current from the first dilution region comprises applying a fifth current between the first dilution region and a first waste reservoir in fluid communication with the first dilution region, the fifth current being substantially opposite to the portion of the third current, so that the portion of the third current is tapped from the first dilution region.

19. The method of claim 18, wherein the tapping step results in a transport of a portion of the first diluted material from the first dilution region into the first waste reservoir.

20. A method for the *in situ* concentration of a material in a microscale channel, comprising:

introducing a first fluid containing said material into a microscale channel to provide a first fluid region in said channel, wherein said first fluid has a first conductivity, and wherein said first fluid region is bounded by second and third fluid regions, said second and third fluid regions having a second conductivity, said second conductivity being greater than said first conductivity; and

applying a voltage gradient along a length of said microscale channel whereby said first, second and third fluid regions are transported along said length of said channel with a first electroosmotic mobility, and whereby said material has an

electrophoretic mobility different from said first electroosmotic mobility whereby said material is concentrated at or near one end of said first fluid region.

21. A microfluidic system for diluting a material in a microfluidic device, the system comprising:

a microfluidic device having at least a first main channel disposed therein, said main channel having at least one microscale cross-sectional dimension;

at least a first source of said material in fluid communication with said main channel at a first point along a length of said main channel;

at least a first diluent source in fluid communication with said main channel at a second point along said length of said main channel;

at least a first reservoir in fluid communication with said main channel at a third point along said length of said main channel;

at least a second diluent source in fluid communication with said main channel at a fourth point along said length of said main channel, said third point being between said second point and said fourth point; and

a fluid direction system for delivering first diluent and material to said main channel from the first source of material and first diluent source, combining said diluent with said material to form first diluted material, for transporting a portion of said first diluted material out of the main channel into the first reservoir, and for introducing second diluent from the second diluent source into the main channel combining the second diluent with the first diluted material in the main channel.

22. The microfluidic system of claim 21, further comprising:

at least a third diluent source in fluid communication with said main channel at a fifth point along said length of said main channel, said fourth point being between said fifth point and said third point; and

a second reservoir in fluid communication with said main channel at a sixth point along said length of said main channel, said sixth point being between said fourth point and said fifth point.

23. The microfluidic system of claim 21, wherein said fluid direction system comprises an electroosmotic fluid direction system.

24. The microfluidic system of claim 21, wherein said fluid direction system comprises:

at least first, second and third electrodes placed in electrical contact with said source of material, said first diluent source and said first reservoir; and

a voltage controller for concurrently applying a voltage at each of said first, second and third electrodes, to deliver material from said source of material and said first diluent source, to said main channel to produce a first diluted material, and continuously transport at least a portion of said first diluted material from said main channel to said first reservoir.

25. The microfluidic system of claim 21, further comprising at least a second reservoir in fluid communication with said main channel at a third point.

26. The microfluidic system of claim 25, further comprising at least a third reservoir in fluid communication with said main channel at a fourth point.

27. The microfluidic system of claim 21, wherein said at least first reservoir comprises a mixing chamber, said mixing chamber having at least one of a depth or width dimension greater than a depth or width dimension of said main channel.

28. The microfluidic system of claim 27, wherein said mixing chamber has a depth dimension that is greater than said depth dimension of said main channel.

29. The microfluidic system of claim 21, wherein said microfluidic device comprises:

a top portion;

a bottom portion; and

an interior portion, the interior portion comprising said main channel, a first diluent channel and a first reservoir channel, said first diluent channel intersecting said main channel at said first point and fluidly connecting said main channel with said first diluent source, and said first reservoir channel intersecting said main channel at said second point and fluidly connecting said first reservoir and said main channel.

30. The microfluidic system of claim 29, wherein said bottom portion comprises a first substrate having at least a first surface, each of said main channel, first diluent channel and first reservoir channel comprise grooves fabricated into said first surface, and said top portion is laminated to said first surface of said first substrate, thereby forming said interior portion disposed between said first surface and the top portion.

31. The microfluidic system of claim 30, wherein each of said source of material, said first diluent source and said first reservoir comprise wells fabricated into said top portion and in fluid communication with said main channel, said first diluent channel and said first reservoir channel, respectively.

32. The microfluidic system of claim 21, wherein said main channel has at least one cross-sectional dimension in the range between 0.1 μm and 500 μm.

33. The microfluidic system of claim 29, wherein at least one of said top portion, said interior portion and said bottom portion are fabricated from a polymeric material.

34. The microfluidic system of claim 29, wherein at least one of said top portion, said interior portion and said bottom portion are fabricated from glass.

35. The microfluidic system of claim 21, wherein said microfluidic device comprises at least two main channels disposed therein, each of said at least two main channels being in fluid communication with a different source of material.

36. The microfluidic system of claim 21, wherein said microfluidic device comprises at least four main channels disposed therein, each of said at least four main channels being in fluid communication with a different source of material.

37. The microfluidic system of claim 21, wherein said microfluidic device comprises at least two main channel disposed therein, each of said at least two main channels being in fluid communication with said at least first source of material.

38. The microfluidic system of claim 21, wherein said microfluidic device comprises at least four main channels disposed therein, each of said at least four main channels being in fluid communication with said at least first source of material.

39. A microfluidic system for continuously diluting a material in a microfluidic device, the system comprising:

a microfluidic device comprising a first channel disposed therein;

a source of said material in fluid communication with said first channel; and

a continuous dilution module, said dilution module comprising:

a diluent source in fluid communication with said first channel at a first point and a first reservoir in fluid communication with said first channel at a second point; and

a fluid direction system for continuously delivering diluent to said first channel to be combined with said material to form first diluted material, and continuously transporting at least a portion of said first diluted material from said first channel to said first reservoir.

40. The microfluidic system of claim 39, wherein said microfluidic system comprises a plurality of dilution modules serially disposed along and in fluid communication with said first channel.

41. The microfluidic system of claim 39, wherein said fluid direction system comprises:

at least first, second and third electrodes placed in electrical contact with said material source, said diluent source and said first reservoir; and

a voltage controller for concurrently applying a voltage at each of said first, second and third electrodes, to continuously deliver diluent to said first channel and continuously transport at least a portion of said first diluted material from said first channel.

42. A microfluidic system comprising:

a first main channel and a second main channel;

a first diluter channel and a second diluter channel, said first and second diluter channels having first and second ends, wherein said first ends of said first and

second channels are in fluid communication with said first main channel, and said second ends of said first and second diluter channels are in fluid communication with said second main channel at said second ends, and wherein said first diluter channel further comprises a diluent reservoir disposed therein;

an electrical control system for applying a voltage gradient between said first main channel and said second main channel.

43. A microfluidic system for concentrating a material in a microfluidic device, comprising:

a microfluidic device having a first channel disposed therein, said first channel having first and second fluid regions disposed therein, said first fluid region comprising said material and having a first conductivity, and said second fluid regions being disposed within said first channel on both ends of said first fluid region and having a second conductivity, said second conductivity being greater than said first conductivity; and

an electroosmotic fluid direction system for transporting said first and second fluid regions along said first channel.

44. The microfluidic system of claim 43, wherein said microfluidic device further comprises:

a source of said first fluid in fluid communication with said first channel

a source of said second fluid in fluid communication with said first channel; and

wherein said electroosmotic fluid direction system selectively injects said first fluid region of material into said first channel and transports said first and second fluid regions through said first channel.

45. The microfluidic system of claim 44, wherein said microfluidic device further comprises:

a material loading channel intersecting and in fluid communication with said first channel; and

a waste channel intersecting and in fluid communication with said first channel.

46. The microfluidic system of claim 45, wherein each of said material loading channel and said waste channel includes a region having a depth less than a depth of said main channel.

47. The microfluidic system of claim 46, wherein each of said regions has a depth less than about one fifth a depth of said first channel.

48. The microfluidic system of claim 45, wherein said second conductivity is at least twice as great as said first conductivity.

49. The microfluidic system of claim 45, wherein said second conductivity is at least five times greater than said first conductivity.

50. The microfluidic system of claim 45, wherein said second conductivity is at least ten times greater than said first conductivity.

51. In a microfluidic device in which first and second currents are applied to a first fluid region, the total of the first and second applied currents exceeding a desired maximum total current, a method of maintaining a current in the first fluid region below the desired maximum total current, comprising applying at least a third current to the first fluid region sufficient to reduce the current in the first fluid region to below the desired maximum current.

52. The method of claim 51, wherein the third current is applied at a point in the first fluid region between a point in the first fluid region at which the first current is applied and a point in the first fluid region at which the second current is applied.

53. The method of claim 51, wherein the third current is substantially opposite to at least one of the first and second currents.

54. The method of claim 51, wherein the first current comprises an aggregate of fourth and fifth currents, the fourth and fifth current causing movement of a first material and a first diluent into the first fluid region.

55. The method of claim 51, wherein the first current comprises an aggregate of fourth and fifth currents, the fourth current being applied at a first reservoir containing a first material and the fifth current being applied at a first diluent reservoir, each of the first material reservoir and first diluent reservoir being in fluid communication with the first fluid region, and the fourth and fifth currents causing a movement of the first material and first diluent into the first fluid region, respectively, to produce a first diluted material.

56. The method of claim 55, wherein the third current is applied to a first waste reservoir that is in fluid communication with the first fluid region.

57. The method of claim 56, wherein the third current causes movement of at least a portion of the first diluted material from the first fluid region into the first waste reservoir.

58. The method of claim 51, wherein the desired total maximum current in the first fluid region is less than about 10 mA.

59. The method of claim 51, wherein the desired total maximum current in the first fluid region is less than about 1 mA.

60. The method of claim 51, wherein the desired total maximum current in the first fluid region is less than about 100 μA.

61. The method of claim 51, wherein the desired total maximum current in the first fluid region is less than or equal to about 20 μA.

62. The method of claim 51, wherein the desired total maximum current in the first fluid region is less than or equal to about 10 μA.

63. The use of a microfluidic device for performing in situ dilution of a first material, the device comprising a first dilutiuon region in fluid communication with a source of the material, first and second diluent sources in fluid commnication with the dilution region, and a fluid transport system for selectively delivering the first material and first diluent to the first dilution region to produce a first diluted material, for transporting a portion of the first diluted material out of the first dilution region and for transporting the second diluent to the first dilution regoion to create a second diluted material.

64. The use of the system of any one of claims 21, 39, 42 or 43.

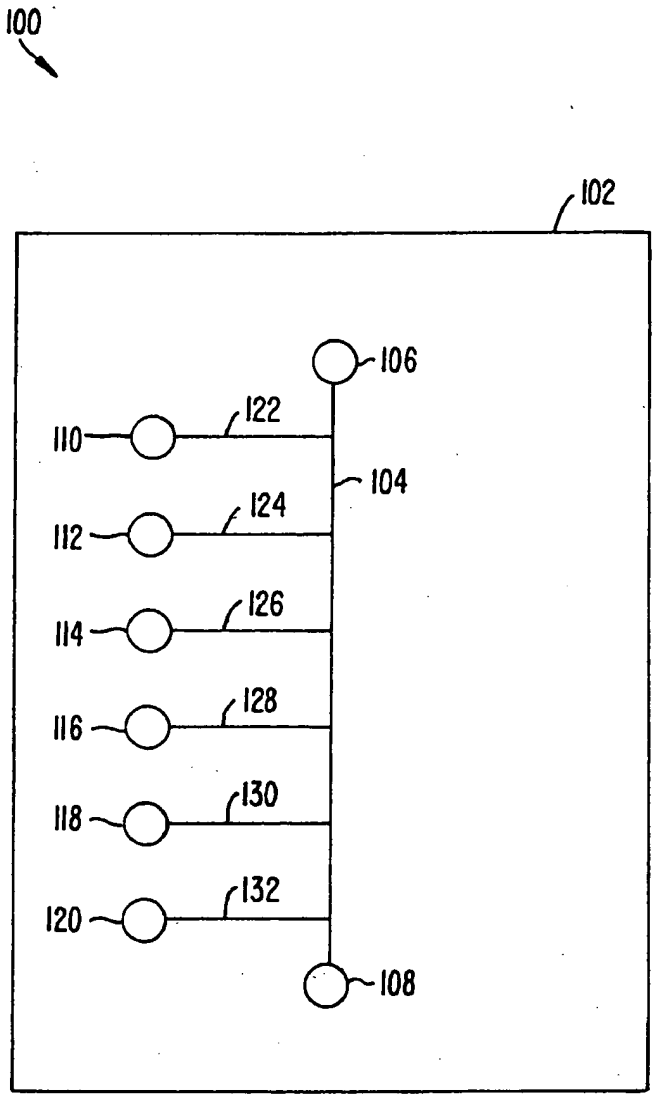

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FIG. 1.

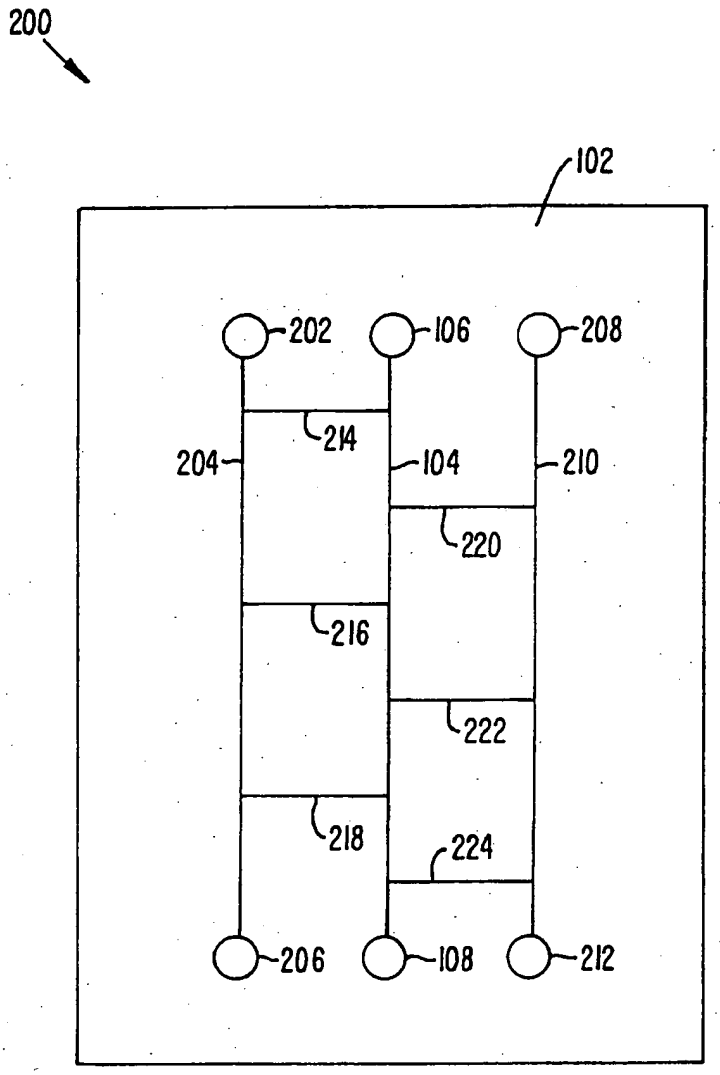

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FIG. 2.

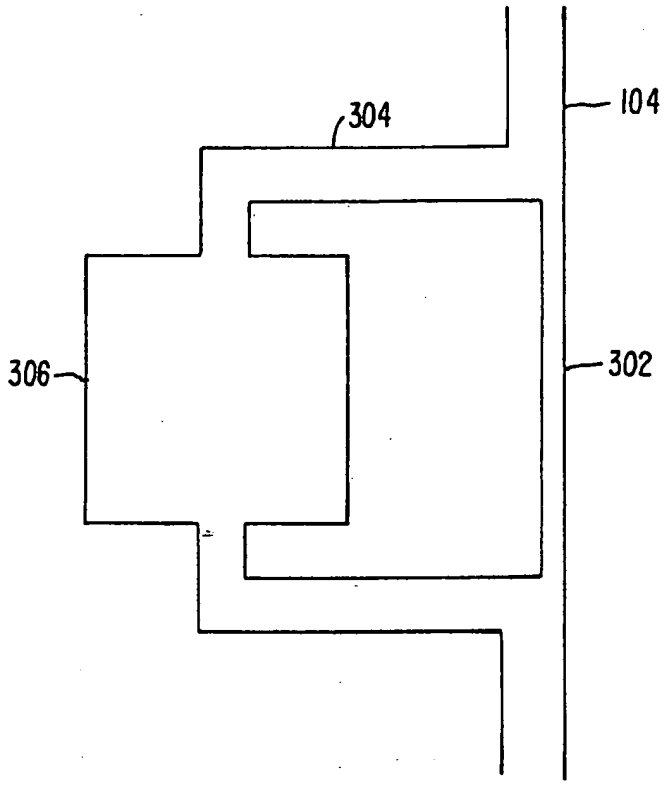


FIG. 3.

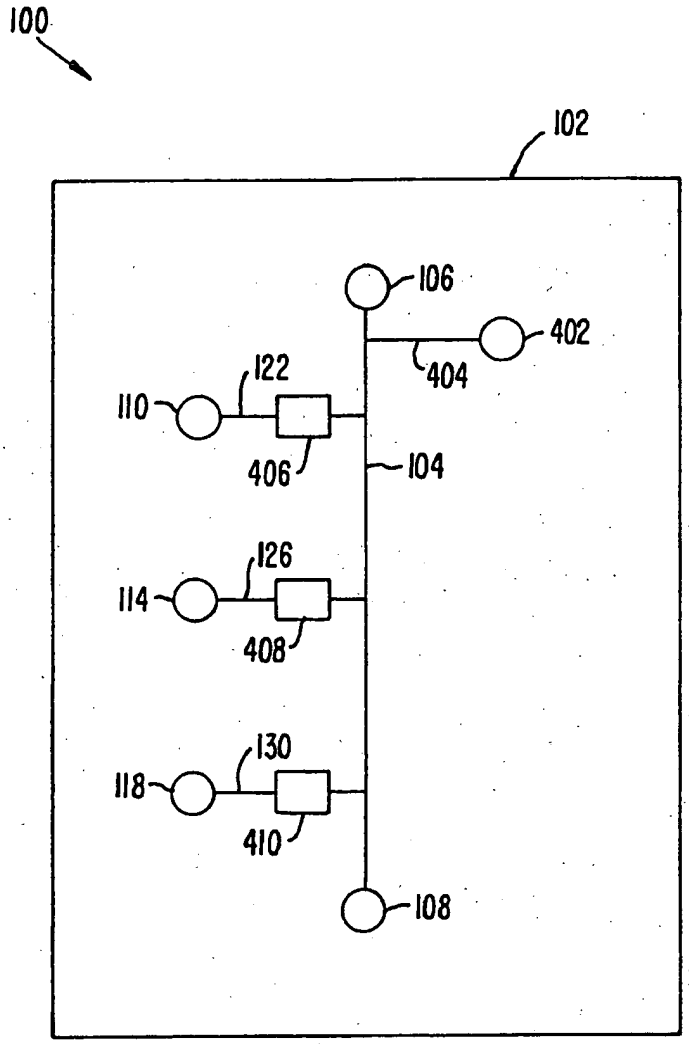


FIG. 4.

FIG. 5A.

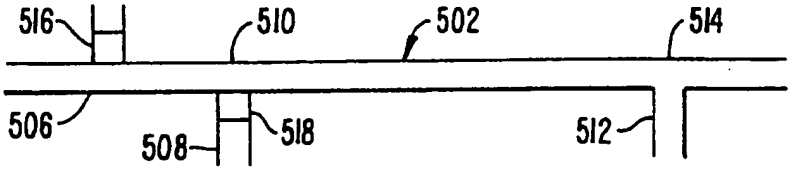


FIG. 5B.

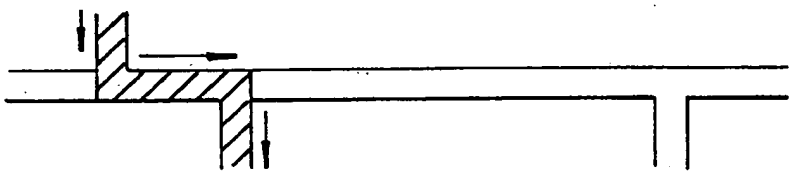


FIG. 5C.

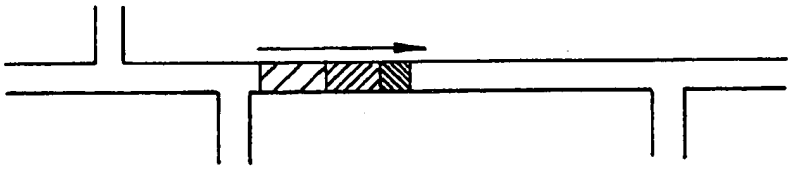


FIG. 5D.

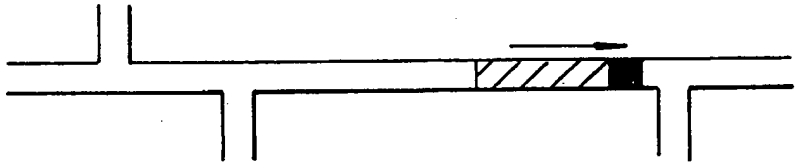
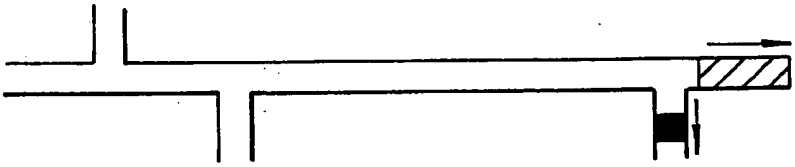


FIG. 5E.



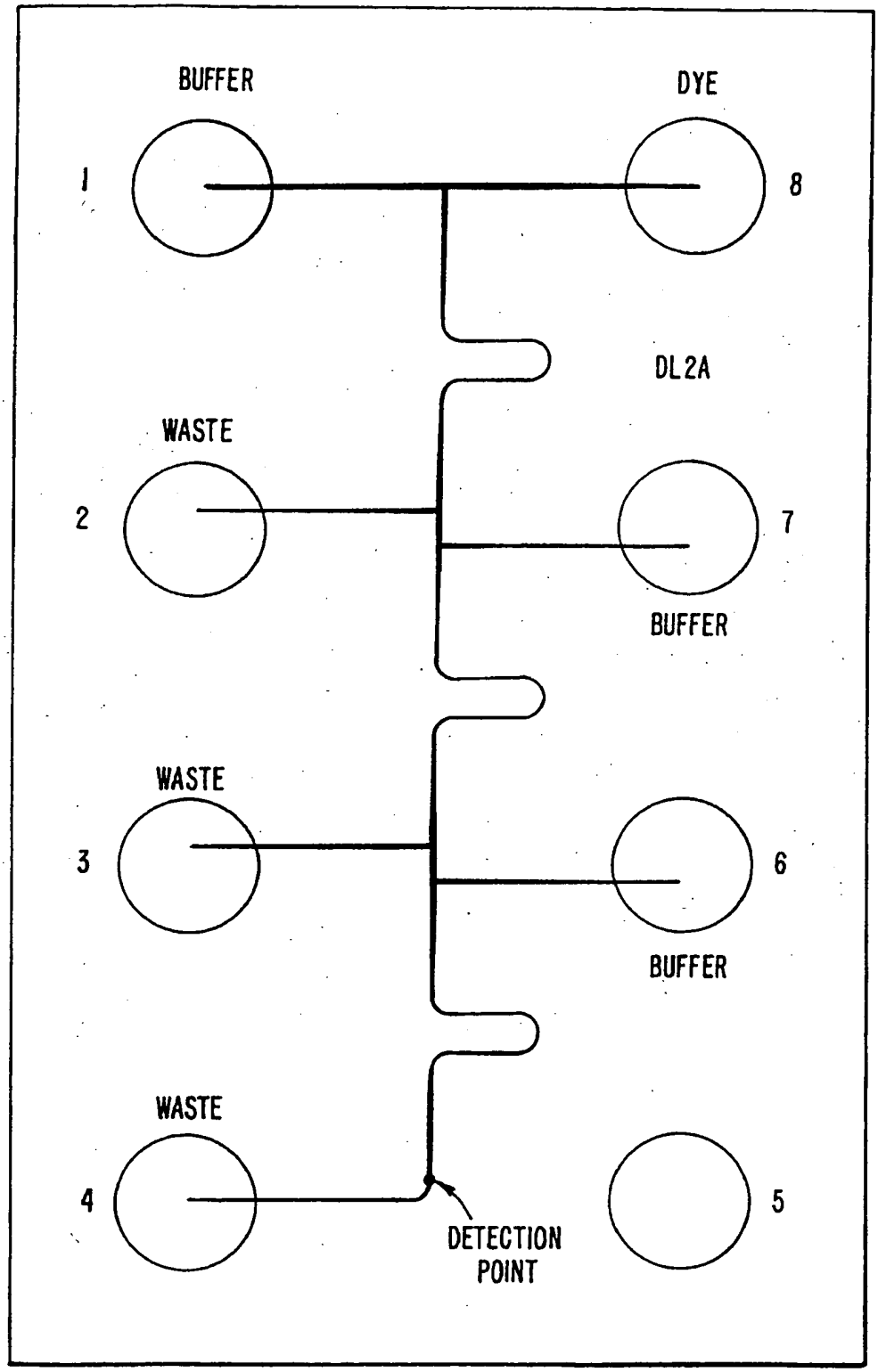

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FIG. 6.

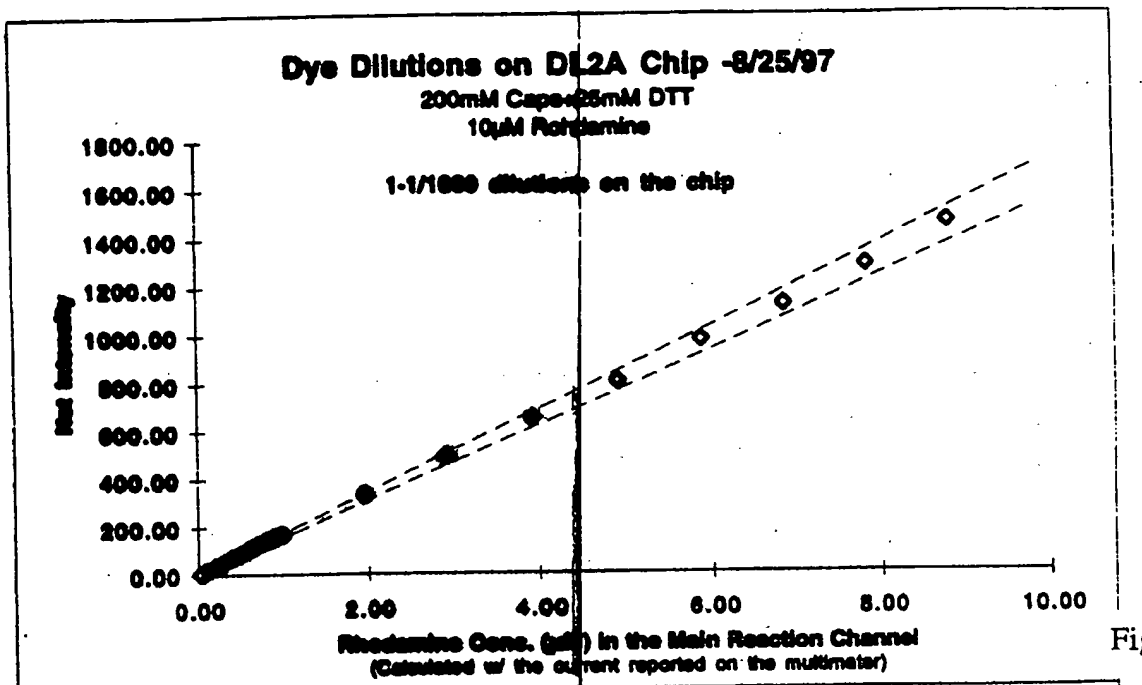


Figure 7A

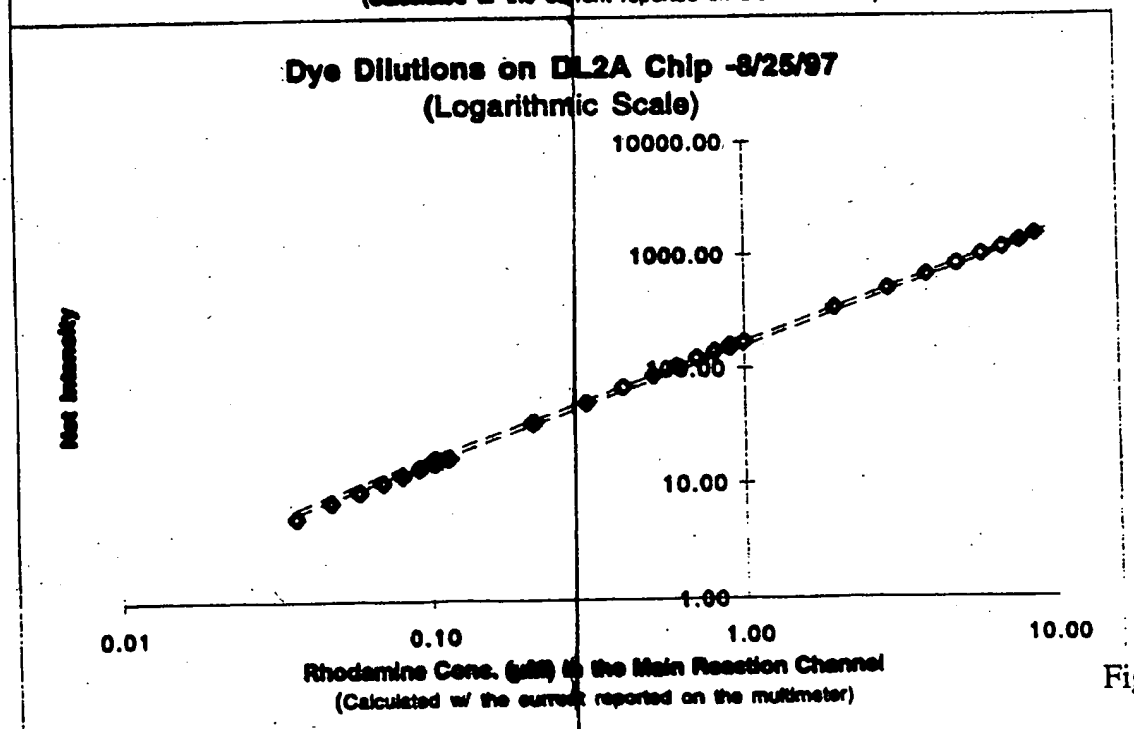


Figure 7B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/12127

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01L 3/00, 11/00

US CL :422/100, 68.1, 58; 436/52, 179, 180; 73/863; 204/600

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/100, 68.1, 58; 436/52, 179, 180; 73/863; 204/600

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN

search terms: Parce Wallace, microfluidic, electroosmotic, dilut?, concentr?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,036,062 A (CRUZAN) 19 July 1977, entire document, especially column 1.	1-64
Y	US 4,079,913 A (ROOF) 31 January 1978, whole document, especially column 1.	1-64
Y	US 5,391,274 A (SHIEH) 21 February 1995, whole document, especially columns 2-3.	1-64
Y	MANZ et al. Electroosmotic pumping and elestrophoretic separations for miniaturized chemical analysis systems. Journal of Micromechanical Microengineering. 1994, Vol. 4, pages 257-265.	1-64

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
31 AUGUST 1998	02 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FARIBORZ MOAZZAM Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet)(July 1992)★